J. Steroid Biochem. Molec. Biol. Vol. 39, No. 5B, pp. 839–850, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

THE ORIGIN AND SIGNIFICANCE OF 18-HYDROXYCORTISOL: STUDIES IN HYPERALDOSTERONISM AND IN BOVINE ADRENOCORTICAL CELLS *IN VITRO*

R. FRASER,[1]* J. M. C. CONNELL,[1] P. S. BUDD,[2] J. E. T. CORRIE[2] and C. J. KENYON[1]
[1]MRC Blood Pressure Unit, Western Infirmary, Glasgow G11 6NT and [2]MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 2XU, Scotland

Summary—18-Hydroxycortisol has been suggested as a marker compound for a transitional zone between the adrenocortical zonae glomerulosa and fasciculata. The control of secretion of 18-hydroxycortisol has been compared with those of cortisol and aldosterone in normal subjects and patients with primary hyperaldosteronism. Comparisons were also made in isolated bovine zona glomerulosa and zona fasciculata cell preparations. Although there was considerable cross-contamination between fractions, 18-hydroxycortisol secretion occurred with equal facility in both fractions but depended on the availability of cortisol as substrate. Changes in secretion during stimulation following those of cortisol. It is concluded that, *in vivo*, 18-hydroxycortisol derives mainly from the zona fasciculata. The relevance of these findings to primary hyperaldosteronism and to the nature of the transition is discussed.

INTRODUCTION

The mammalian adrenal cortex consists of three layers, the zona glomerulosa immediately beneath the adrenal capsule, the zona fasciculata and zona reticularis which is adjacent to the adrenal medulla. These zones have different biosynthetic capacities. The zona glomerulosa synthesises aldosterone (by 18-hydroxylating corticosterone) but not cortisol (which requires a 17α-hydroxylation reaction). The converse is true of the zonae fasciculata and reticularis, although these zones are capable of 18-hydroxylating several other compounds, particularly 11-deoxycorticosterone (DOC).

Current opinion suggests that adrenocytes originate in the outer adrenal cortex and migrate inwards [1]. Two models exist. In the first, cells are initially of the zona glomerulosa type but during migration lose the ability to synthesize aldosterone but gain that to synthesize cortisol, possibly passing through an intermediate transition state. In the second model, a "germ layer" produces cells which become either glomerulosa or fasciculata type. In either case, the transition state or the "germ" layer, the component cells may be pluripotent, capable of both aldosterone and cortisol synthesis.

In 1982, Chu and Ulick [2] isolated and identified a novel steroid compound, 18-hydroxycortisol, from human urine which, with the related compound 18-oxocortisol, has been shown to originate from the adrenal cortex [3]. It has been postulated that these "hybrid" corticosteroids, with both 18 and 17α oxygen functions, might be markers of the pluripotent cells mentioned above [4, 5] and that the distribution of their synthesis within the adrenal cortex should be concentrated in the outer cell layers.

A study of this distribution should therefore be of value in understanding the progressive functional changes in the cell during migration. In this context, it is also relevant that 18-hydroxycortisol secretion is also markedly increased in two rare forms of endocrine hypertension, primary hyperaldosteronism due to an adrenocortical adenoma (Conn's syndrome) and dexamethasone suppressible hyperaldosteronism, but not in idiopathic hyperaldosteronism [3, 6–8]. There follows a brief outline of the control of cortisol, aldosterone and 18-hydroxycortisol secretion in normal subjects and in hyperaldosteronism. A more detailed discussion is available [9]. The distribution and control of 18-hydroxycortisol secretion has then been studied in bovine adrenocortical cells which

Proceedings of the Symposium on Recent Advances in Steroid Endocrinology, held in honour of Professor V. H. T. James, London, England, 1 November 1990.
*To whom correspondence should be addressed.

synthesize a spectrum of steroids resembling that in man.

Normal control

A very large number of factors have been shown to influence adrenocortical function but the major factors remain ACTH from the anterior pituitary gland, angiotensin II, a pressor octapeptide released in the circulation by the consecutive actions of the renal enzyme renin and an angiotensin-converting enzyme, potassium and sodium status [9]. The pattern of control for cortisol, a glucocorticoid, and aldosterone, a mineralocorticoid, are quite different. That of 18-hydroxycortisol, apparently devoid of biological activity [10], resembles cortisol more closely than aldosterone but with important differences.

Cortisol

Cortisol biosynthesis is controlled by the anterior pituitary peptide hormone, ACTH which, by stimulating adenylate cyclase activity, increases the rate of reactions in the early pathway, particularly the cholesterol side chain cleavage reaction which is catalysed by cytochrome $P450_{SCC}$. There are secondary increases in the rate of reactions later in the pathway e.g. $11\beta/18$ and 17α-hydroxylation and in the concentrations of the non-haem iron/protein, adrenodoxin [11] which is involved in the mixed function oxidase reaction. The increased rate of these hydroxylation reactions follows a rise in the rate of expression of the appropriate cytochrome *P*450 genes as indicated by increased mRNA levels [12]. Longterm excess of ACTH results in sustained high levels of cortisol, of its precursor 11-deoxycortisol and of the constituent compounds of the 17-deoxycorticosterone pathway, DOC, corticosterone and 18-hydroxycorticosterone but it causes only a transient increase in aldosterone secretion; secretion then falls to very low levels [13] and zona glomerulosa volume becomes progressively reduced [14, 15].

Aldosterone

The major factors in the control of aldosterone biosynthesis are angiotensin II and potassium. Plasma renin concentration, and consequently that of angiotensin II, rises during sodium depletion and is suppressed by high sodium intake with plasma aldosterone levels following suit. Angiotensin II within the physiological range affects only aldosterone and 18-hydroxycorticosterone secretion. It does not increase plasma cortisol concentration; indeed there have been reports of a mild inhibitory action [16]. However, it is a potent stimulus to cortisol secretion *in vitro* (see Discussion).

The peptide probably stimulates the activity of both early and late (i.e. conversion of corticosterone to aldosterone) pathways. Current opinion is that activation of angiotensin II receptors in the zona glomerulosa cell membrane decreases permeability to potassium ions, leading to membrane depolarization and the consequent opening of voltage-dependent calcium channels with calcium influx. Simultaneously, hydrolysis of phosphatidyl inositol yields inositol triphosphate which encourages the release of intracellular bound calcium ions, contributing to the rise in intracellular free calcium, and diacylglycerol which activates protein kinase C. Increased free calcium ion concentration and kinase activity eventually lead to increased aldosterone synthesis although the intermediate reactions are not understood. Rising intracellular free calcium concentrations also results in the opening of calcium-dependent potassium channels, restoring potassium permeability and thence cell membrane potential. These complex events have been discussed in detail elsewhere [17, 18].

Potassium probably also acts by depolarizing the cell membrane. Potassium causes an increase in plasma aldosterone concentration in direct relation to changes in plasma potassium although stimulation may occur at potassium doses insufficient to alter circulating potassium [19]. It does not affect cortisol secretion.

Sodium intake not only alters basal aldosterone but modulates its responsiveness to other agonists. Thus, the acute response of plasma aldosterone concentration to ACTH, angiotensin II and potassium is increased in sodium-deplete subjects and decreased in sodium loaded subjects [20, 21]. The effect of potassium intake is the reverse of this. Again, the mechanism of this sensitizing effect of electrolyte status is not clear but several mediating factors have been suggested.

Dopamine infusion into sodium-deplete subjects at a constant rate reduce the level of aldosterone response to approximately that found in sodium replete subjects [22]. This finding, together with the actions of dopamine agonist and antagonist drugs, led to the hypothesis that aldosterone secretion is under tonic inhibition by dopamine which is relieved during

sodium depletion. Unfortunately, the dopamine effect is demonstrable only at very high dose rates, at which the angiotensin II clearance rate may itself be altered [23]. Moreover, dopamine has no effect on the response of aldosterone to ACTH [24]. The "dopamine hypothesis" has been discussed by several authors [25–27].

A similar role has been invoked for atrial natriuretic peptide (ANP). ANP inhibits aldosterone biosynthesis *in vitro* and *in vivo*; responses to angiotensin II and ACTH are both said to be affected although the anti-ACTH effect may be equivocal. Thus Cuneo *et al.* [28] infused ANP at "physiological" rates into normal human subjects on low sodium intake and showed a fall in the aldosterone response to angiotensin II but not to ACTH. They discuss the importance of ANP in the control of adrenocortical function and electrolyte balance. Finally, several groups have suggested that prolactin might alter aldosterone responses to angiotensin II (e.g. see [29]); as far as we are aware, no equivalent information on ACTH is available.

18-Hydroxycortisol

Control of 18-hydroxycortisol secretion rate follows a pattern resembling that of cortisol more closely than aldosterone. Thus, dexamethasone, a synthetic glucocorticoid which inhibits ACTH secretion, causes a fall in the level of 18-hydroxycortisol in plasma and urine while short-term ACTH infusion has the opposite effect [30–32] (Fig. 1). These increases, unlike those of aldosterone, are sustained during chronic ACTH excess [33]. Consequently, 18-hydroxycortisol levels correlate closely and positively with those of cortisol, not aldosterone. Curiously, sodium depletion causes a small increase in plasma and urine 18-hydroxycortisol in most, but not all, subjects (Fig. 2) but infusion of angiotensin II, the usual mediator of adrenocortical responses to sodium status, is without effect [30–32] (Fig. 3). The mechanism of this effect remains obscure.

Control in primary hyperaldosteronism

Primary hyperaldosteronism is characterized by a high basal secretion of the mineralocorticoid from an adrenocortical lesion which results in suppression of plasma renin concentration, hypokalaemia, increased total body or exchangeable sodium, a metabolic alkalosis and hypertension. Three main subcategories are recognizable, primary hyperaldosteronism due to a benign adrenocortical adenoma (Conn's syndrome), idiopathic hyperaldosteronism due to bilateral adrenocortical micronodular hyperplasia and dexamethasone-suppressible (≡glucocorticoid-remediable) hyperaldosteronism (for review see [34]). Idiopathic hyperaldosteronism will not be considered further.

In Conn's syndrome, aldosterone secretion is much more dependent upon ACTH than in normal subjects. Thus, it follows the well-documented circadian rhythm characteristic of

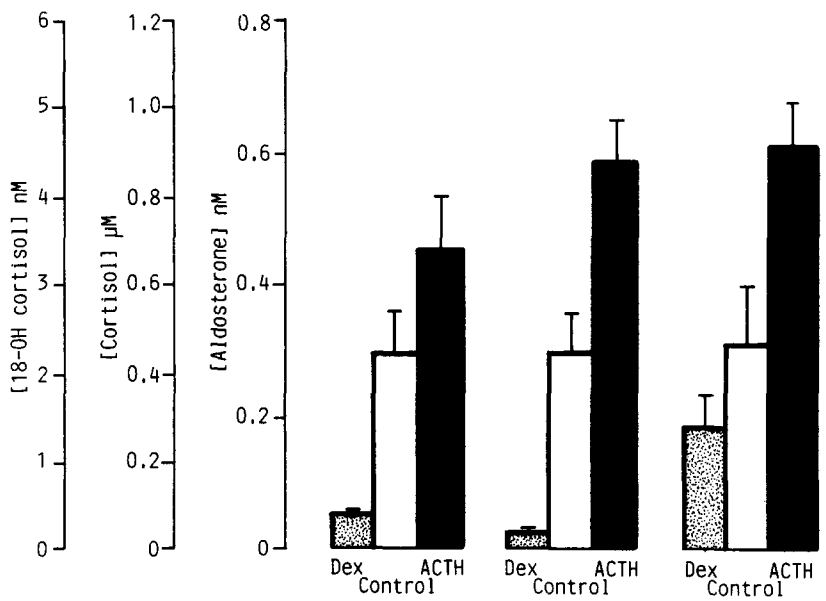

Fig. 1. Effect on plasma corticosteroid concentrations of ACTH (250 μg Synacthen, i.v.) and dexamethasone (2 mg/day for 2 days) in normal male subjects ($n = 6$) on normal sodium intake (mean ± SE; redrawn from Corrie *et al.* [30]).

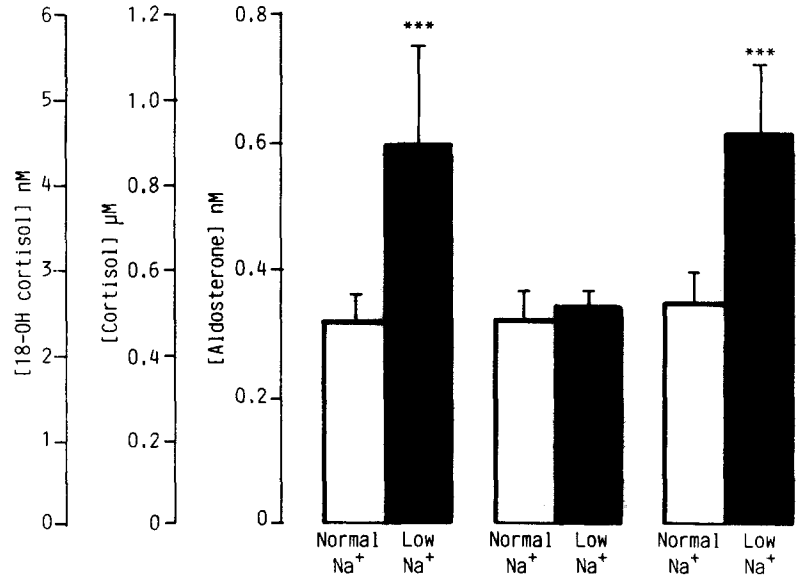

Fig. 2. Effect on plasma corticosteroid concentrations of dietary sodium intake in normal male subjects ($n = 6$, mean ± SE). Normal intake was *ad libitum*; low sodium intake was 20 mmol/day for 1 week (redrawn from Corrie *et al.* [30]).

cortisol and responds vigorously to ACTH infusion. However, dexamethasone, while causing some inhibition, does not return plasma aldosterone concentration to normal, showing that a considerable component of secretion is autonomous. In contrast, angiotensin II infusion does not increase plasma aldosterone concentration and, at higher dose rates, may be inhibitory [35]. Interestingly, administering a low, constant rate of ACTH simultaneously with graded infusion rates of angiotensin II restores the normal dose-response relationship between angiotensin II and aldosterone [36]. To explain the inhibitory action of angiotensin II alone, it was therefore suggested that exogenous peptide might inhibit ACTH secretion; while some evidence of this inhibition has been obtained in normal subjects [37], other studies show a stimulation of ACTH by angiotensin II [38, 39] and the problem remains to be resolved. Following surgical excision of the tumour, the normal angiotensin II/aldosterone relationship is restored.

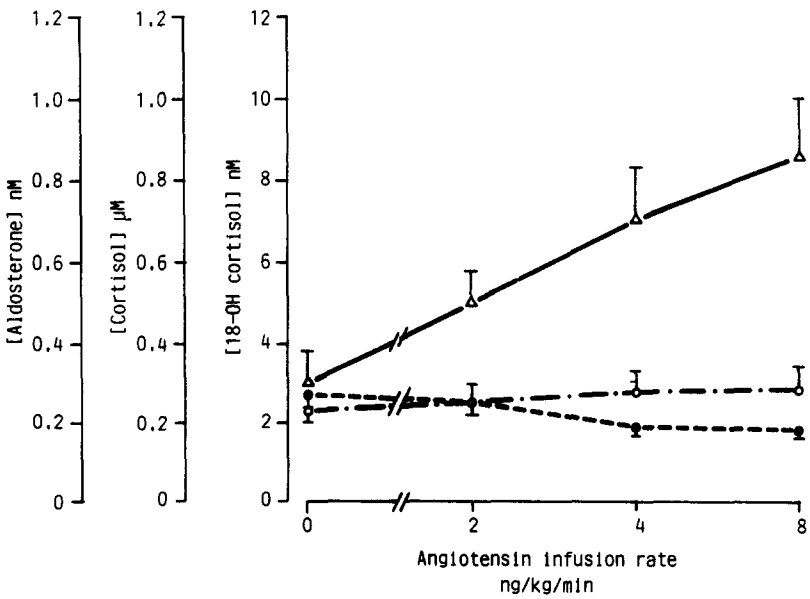

Fig. 3. Effect of angiotensin II infusion on plasma corticosteroid concentration in normal male subjects ($n = 6$, mean ± SE) taking a medium (100 mmol/day for 1 week) sodium intake (redrawn from Corrie *et al.* [30]).

Dexamethasone-suppressible hyperaldosteronism is a familial disease inherited as an autosomal dominant trait. In these patients, small doses of synthetic glucocorticoid suppress plasma aldosterone concentration to low normal or low levels and also correct all biochemical abnormalities and blood pressure. Little is known of the adrenocortical histology in this disease. The control of corticosteroid secretion has been discussed by Connell *et al.* [5] and Fraser [9]. Briefly, as in Conn's syndrome and predictably from the action of dexamethasone, aldosterone secretion is largely, possibly entirely, dependent upon ACTH. Its circadian rhythm is identical to those of other ACTH-dependent steroids and it responds with supranormal sensitivity to infused ACTH. Moreover, the ACTH effect is clearly sustainable long-term. Conversely, there is no clear relationship between plasma aldosterone and angiotensin II during infusion. However, following dexamethasone therapy, during which exchangeable sodium falls to within the normal range, the normal positive relationship is restored, indicating that the original insensitivity to angiotensin II was due to excess sodium retention (see above) and not to some intrinsic receptor or post-receptor defect. The supersensitivity to ACTH is not due to reduced dopamine tonic inhibitory action (see previous discussion) since infusions of the catecholamine do not attenuate the effect of infused ACTH; ANP levels and their response to exercise are not subnormal but the effects of concurrent ANP infusion have not been tested.

As mentioned in the Introduction, these types of hyperaldosteronism have in common the secretion of abnormally high rates of 18-hydroxycortisol, secretion of which is clearly dependent on ACTH and, in dexamethasone-suppressible hyperaldosteronism, is suppressed by the glucocorticoid.

There has been much speculation concerning the precise genetic abnormality and its relevance to normal adrenocortical function. It has been postulated that 18-hydroxycortisol, as a corticosteroid with characteristics of both zona glomerulosa and zona fasciculata products (see above), should be synthesized only at the interface between these zones. Therefore, it follows, that in the two forms of primary hyperaldosteronism described, this interface must be abnormal. However, the restricted distribution of 18-hydroxycortisol biosynthesis has not been unequivocally established. The following studies have addressed this question.

EXPERIMENTAL

Materials

Materials were obtained from the following sources: Medium 199 and collagenase (Flow Laboratories, Irvine, Strathclyde), nylon gauze (Henry Simon, Stockport, Cheshire), ACTH ('Synacthen'; CIBA, Horsham, Sussex), dibutyryl cyclic AMP, 12-*O*-tetradecanoylphorbol acetate (TPA) and A23187 (Sigma Chemical Co., Poole, Dorset), Percoll (Pharmacia Fine Chemicals AB, Uppsala, Sweden), angiotensin II (Cambridge Research Biochemicals, Cambridge, England). Radioactively-labelled cortisol (Corning, MA, U.S.A.) and aldosterone (Amersham Int., Bucks), cortisol antiserum (Scottish Antibody Production Unit). The aldosterone antiserum was a generous gift from Professor Th. J. Benraad (University of Nimegen, The Netherlands).

Methods

Fresh bovine adrenal glands were obtained from a local slaughterhouse. Outer slices (zona glomerulosa) and inner slices (zona fasciculata) were taken and cells prepared and incubated by a modification of the method of Haning *et al.* [40] as described previously [17, 41]. Where appropriate, cell fractions were further fractionated on a discontinuous Percoll gradient with concentrations of Percoll of 5, 10 and 15%. Cortisol and aldosterone radioimmunoassays have also been described previously [17, 41].

RESULTS

Purity of cell preparation

Distinction between zona glomerulosa and fasciculata cells by light microscopy in dispersed cells is less easy in bovine than in rat tissue. An estimate of cross-contamination between preparations was derived from the cortisol secretion rate of the zona glomerulosa preparations and the aldosterone secretion rate of the zona fasciculata preparations. On this basis, the zona glomerulosa contained up to 33% zona fasciculata cells and the zona fasciculata approx. 8% zona glomerulosa cells (Table 1). Sub-fractionation of the zona glomerulosa preparation did not significantly reduce zona fasciculata contamination using this criterion (Table 2). The

Table 1. Basal corticosteroid secretion by bovine adrenocortical cell fractions (ng 10^6 cells h ± SE)

Zone	Glomerulosa	Fasciculata
Cortisol (F)	69.9 ± 3.3	189.5 ± 3.6
18-HydroxyF	5.6 ± 0.3	6.5 ± 0.3
Aldosterone	3.3 ± 0.2	0.24 ± 0.02

Table 2. Effect of percoll gradient fractionation on corticosteroid production by a bovine zona glomerulosa dispersed cell preparation (ng 10^6 cells h ± SE)

% Percoll	5	10	15
Cortisol	24.9 ± 2.2	25.6 ± 2.2	25.8 ± 29
Aldosterone	0.36 ± 0.06	0.39 ± 0.11	0.27 ± 0.06
18-OHF	0.34 ± 0.04	0.39 ± 0.03	0.32 ± 0.03

18-OHF, 18-hydroxycortisol.

secretion rate of 18-hydroxycortisol was not significantly different between zones, nor was it altered by Percoll separation.

Corticosteroid response to ACTH, angiotensin II and potassium

In zona glomerulosa cells, the lowest dose of ACTH (2×10^{-12} M) significantly increased both aldosterone and 18-hydroxycortisol secretion (Fig. 4). The 18-hydroxycortisol response was maximal at 10^{-11} M ACTH but a further increase in aldosterone secretion occurred at the highest dose (5×10^{-11} M). Similarly, zona fasciculata cortisol and 18-hydroxycortisol secretion rose in response to 2×10^{-12} M ACTH but no further increase occurred at higher doses. The maximum 18-hydroxycortisol response was similar in magnitude in both preparations.

Angiotensin II also stimulated 18-hydroxycortisol secretion with similar potency in both preparations (Fig. 5). Significant stimulation was achieved with 10^{-9} M angiotensin II and maximum response had not been reached at 2.5×10^{-8} M. A similar pattern of response was seen for aldosterone and for cortisol in zona glomerulosa and zona fasciculata, respectively.

In zona glomerulosa cells, increasing potassium concentration from 3.8 (basal) to 6.1 mM significantly increased aldosterone secretion but further increases had no effect (Fig. 6). Stimulation of 18-hydroxycortisol required higher potassium levels (8.4 mM). Zona fasciculata cells responded with similar sensitivity but 10.7 mM potassium caused a greater increase in the secretion rates of both 18-hydroxycortisol and cortisol.

The relationships between 18-hydroxycortisol secretion and aldosterone in the zona glomerulosa or cortisol in the zona fasciculata are illustrated in Figs 7 and 8. In both cases, there was a highly significant correlation.

The close physiological correlation between cortisol and 18-hydroxycortisol secretion is further illustrated by Fig. 9 which shows the similarity of their proportionate responses to a variety of agonists including ACTH, angiotensin II and potassium. Thus, significant positive effects were seen for both compounds from exposure to cAMP, the phorbol ester TPA and a calcium ionophore, A23187.

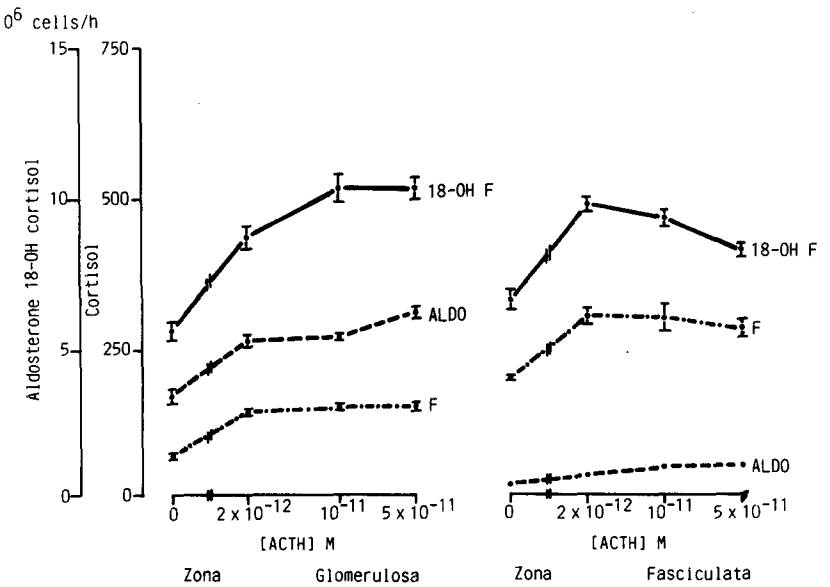

Fig. 4. Response to 18-hydroxycortisol (18-OHF), cortisol (F) and aldosterone (ALDO) secretion in bovine zona glomerulosa and zona fasciculata preparation before and during incubation with ACTH ($n = 5$, mean ± SE).

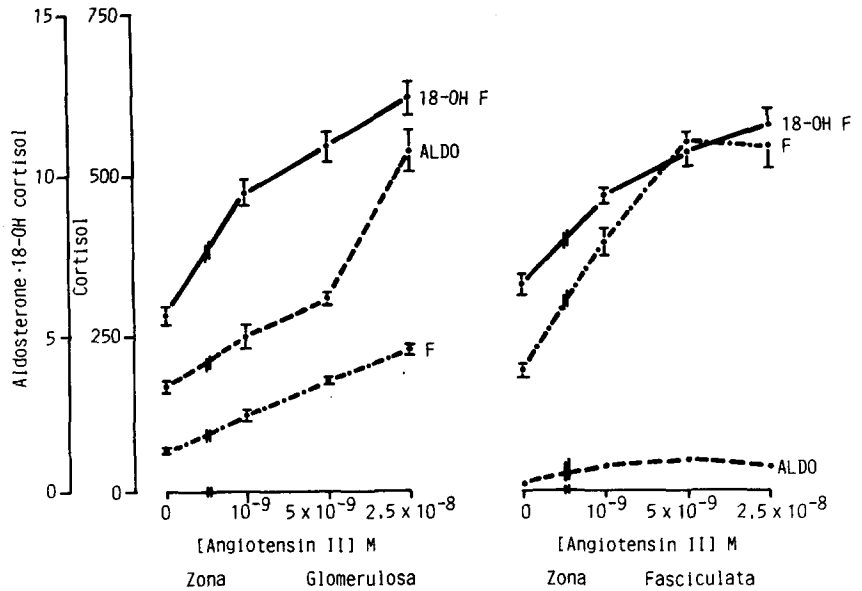

Fig. 5. Responses of 18-hydroxycortisol (18-OHF), cortisol (F) and aldosterone (ALDO) secretion in bovine zona glomerulosa and fasciculata preparations before and during incubation with angiotensin II ($n = 5$, mean ± SE).

A further comparison of the patterns of steroid secretion from zona glomerulosa and zona fasciculata is shown in Table 3. Although the zona glomerulosa fraction secreted more aldosterone, corticosterone and 18-hydroxycorticosterone than the zona fasciculata and these compounds responded with higher capacity to ACTH and angiotensin II in the zona glomerulosa, it is again clear from cortisol secretion rates that there was gross cross-contamination between fractions. Both fractions produced cortisol at a similar rate and were only distinguished by the greater zona fasciculata response to ACTH. Basal 18-hydroxycortisol secretion and its response to ACTH and to angiotensin II were indistinguishable between zones.

There was no difference in the capacity of the zona glomerulosa and zona fasciculata fractions to convert added cortisol to 18-hydroxycortisol (Fig. 10). In both cases, secretion increased approx. 100 fold for an increase in cortisol concentration from 10^{-7} to 10^{-4} (i.e. 1000 fold).

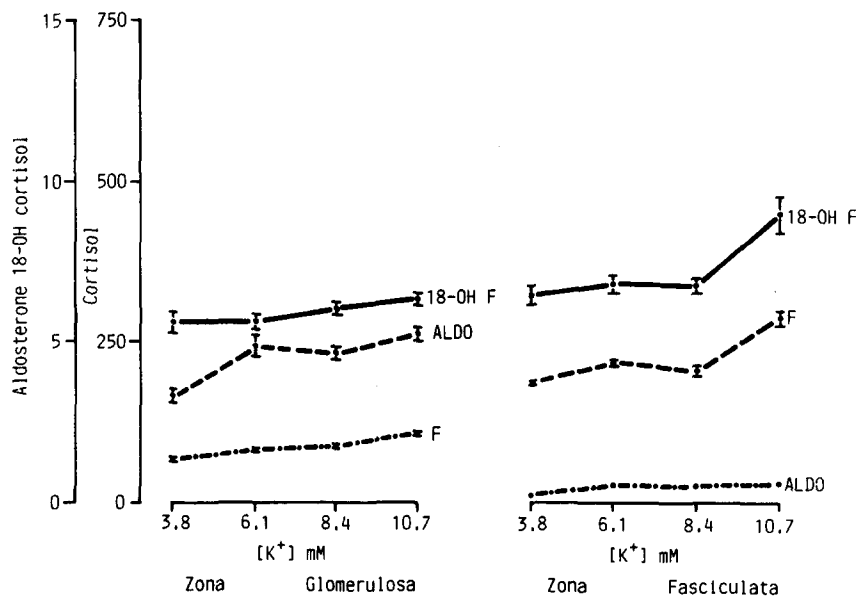

Fig. 6. Responses of 18-hydroxycortisol (18-OHF), cortisol (F) and aldosterone (ALDO) to increasing extracellular potassium concentration in bovine zona glomerulosa and zona fasciculata preparations (mean ± SE).

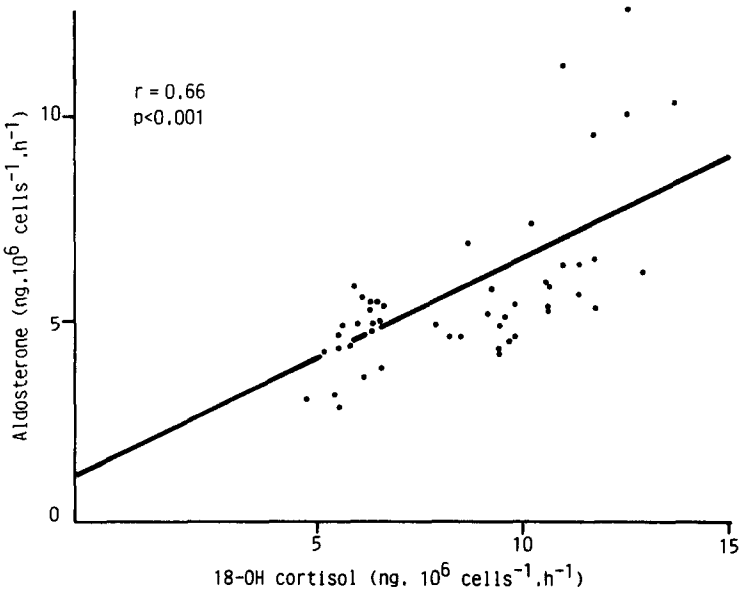

Fig. 7. Correlation of 18-hydroxycortisol and aldosterone secretion in a bovine zona glomerulosa preparation.

DISCUSSION

Although biologically inactive and present in low concentrations in normal plasma, the identification of 18-hydroxycortisol has excited considerable interest for two reasons. Firstly, it has an apparently "hybrid" structure. Secondly, abnormally large quantities are present in the urine and plasma of patients with primary hyperaldosteronism due to an adenoma or dexamethasone-suppressible hyperaldosteronism. Interestingly, in both these syndromes, aldosterone secretion is much more dependent on ACTH and much less dependent on the renin–angiotensin system than in normal subjects and this might provide an important clue to the normal origin of 18-hydroxycortisol. Moreover, histologically, the cells of

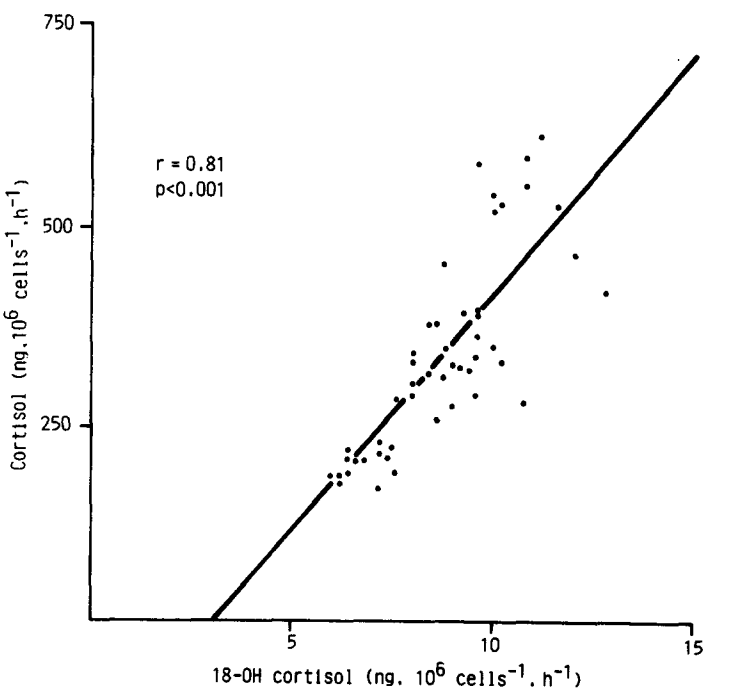

Fig. 8. Correlation of 18-hydroxycortisol and cortisol secretion in a bovine zona fasciculata preparation.

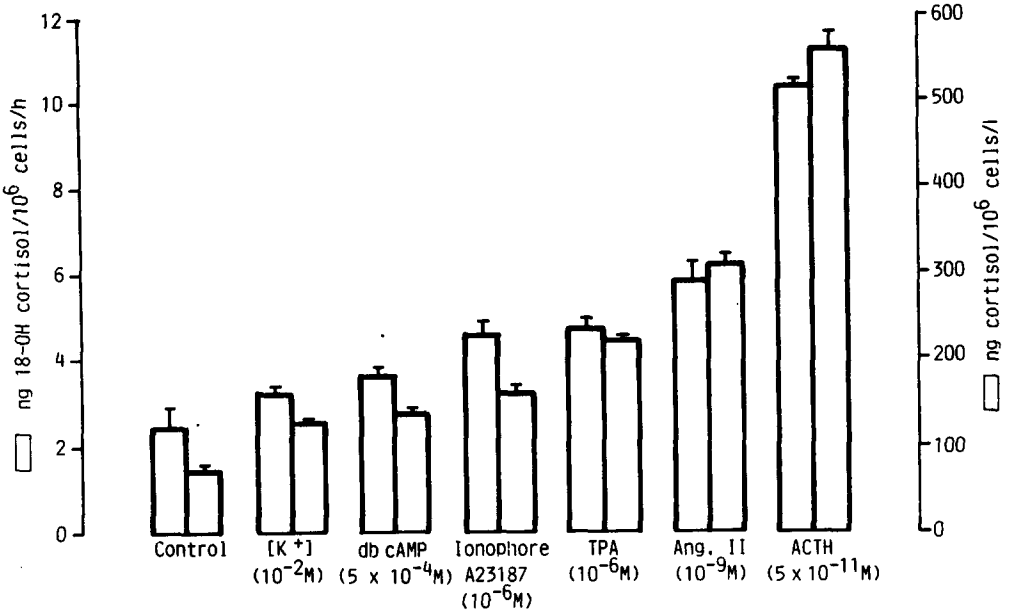

Fig. 9. The effect of various agonists on 18-hydroxycortisol and cortisol secretion from a bovine zona fasciculata preparation (mean ± SE).

aldosterone-producing adenomata resemble zona fasciculata more than zona glomerulosa cells and synthesize significant quantities of cortisol as well as aldosterone [3].

There is general agreement in the literature (see Introduction) that 18-hydroxycortisol secretion responds vigorously to ACTH and is suppressed by dexamethasone. Angiotensin II fails to increase *in vivo* secretion but the response to sodium depletion is equivocal. This pattern of control corresponds more closely to zona fasciculata than zona glomerulosa.

The problem of the intra-adrenal locus of 18-hydroxycortisol synthesis should be more accessible through *in vitro* studies. In an early study, Ulick and Chu [3] showed that cortisol but not corticosterone was converted to 18-hydroxycortisol by slices of bovine adrenal tissue, implying that 17-hydroxylation must precede 18-hydroxylation. In the current study, an attempt has been made to study bovine zona fasciculata and zona glomerulosa tissue separately. In the zona glomerulosa preparation, both ACTH and angiotensin II caused positively correlated increases in cortisol 18-hydroxycortisol secretion. Previous studies have shown angiotensin II as a powerful cortisol agonist *in vitro* (e.g. [41]). Moreover, added cortisol produced a dose-dependent increase in 18-hydroxycortisol secretion, with a dose-response ratio of approx. 10:1, close to that found *in vivo* in human subjects treated with ACTH [3, 8].

In the zona fasciculata fraction, where zona glomerulosa contamination was relatively minor, it is reasonable to assume that most, if not all, the 18-hydroxycortisol originated from the zona fasciculata cells. Interpretation of the data from the zona glomerulosa fraction is more difficult since contamination from zona fasciculata was clearly gross and not improved by further density gradient fractionation. The major proportion of aldosterone was secreted by this fraction but together with significant quantities of cortisol. While aldosterone and 18-hydroxycortisol secretion correlated closely, so did those of cortisol and 18-hydroxycortisol and, *in vitro*, aldosterone and cortisol respond to the

Table 3. Comparison of corticosteroid responses to ACTH (5×10^{-11} M) and angiotensin II (10^{-9} M) in zona fasciculata and zona glomerulosa (ng/10^6 cells/h, $n = 6$)

	Aldosterone	Corticosterone (B)	18-HydroxyB	Cortisol (F)	18-HydroxyF
Zona glomerulosa					
Control	0.58 ± 0.12	10.2 ± 0.6	0.18 ± 0.04	62.6 ± 1.5	1.03 ± 0.23
ACTH	0.91 ± 0.15	29.1 ± 2.5	0.52 ± 0.05	131.7 ± 2.8	2.62 ± 0.09
Angiotensin II	1.18 ± 0.08	36.6 ± 3.7	0.98 ± 0.07	140.4 ± 6.7	2.85 ± 0.13
Zona fasciculata					
Control	0.2 ± 0.02	4.13 ± 0.85	0.13 ± 0.03	53.3 ± 4.3	1.1 ± 0.09
ACTH	0.5 ± 0.06	22.1 ± 2.49	0.32 ± 0.06	216.7 ± 18.1	3.21 ± 0.29
Angiotensin II	0.42 ± 0.04	178.5 ± 0.9	0.44 ± 0.04	141.3 ± 6.6	3.45 ± 0.16

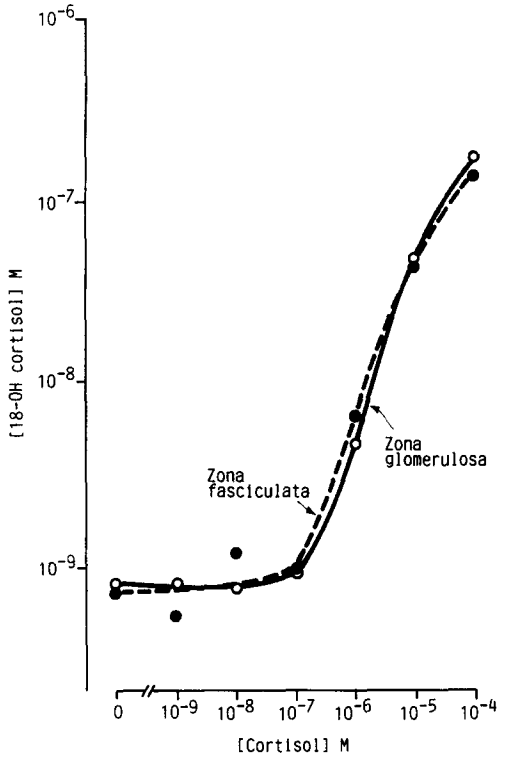

Fig. 10. A comparison of the capacity to convert added cortisol to 18-hydroxycortisol by bovine zona glomerulosa and zona fasciculata preparations.

same agonists. It therefore seems probable that a large proportion, if not all, the 18-hydroxycortisol derived from cortisol secreted by zona fasciculata cells. Whether the subsequent 18-hydroxylation also occurred only in the zona fasciculata cells or both types cannot be determined. Both cell types possess 18-hydroxylase systems capable of synthesizing aldosterone from DOC but they may be different. In bovine and porcine adrenal tissue Yanagibashi *et al.* [42] showed this function to be achieved exclusively by cytochrome $P450_{11\beta/18}$ and that it occurred in both zona glomerulosa and zona fasciculata. However, this is probably not the case in all species. Ogishima *et al.* [43] were able to isolate an enzyme, cytochrome $P450_{aldo}$ from rat zona glomerulosa tissue clearly distinguishable from the zona fasciculata cytochrome $P450_{11\beta/18}$. Similarly, in a series of studies (see [44]), Müller *et al.* demonstrated the presence of two cytochrome $P450_{11\beta/18}$ enzymes in the rat adrenal by different molecular weights. Only the lower molecular weight enzyme content of the zona glomerulosa was affected by increasing potassium, an aldosterone-specific stimulus. However, the mRNAs for these enzymes could not be distinguished by cDNA/probes or electrophoresis and the authors postulate a post-translational influence of potassium converting higher to lower molecular weight species. Other authors (e.g. [45]) suggest separate genes but with very high homology. At present it is not known whether man possesses one or two enzymes, nor are their relative affinities for cortisol known. However, zona glomerulosa cells are unlikely to be exposed to more than peripheral plasma concentrations of cortisol *in vivo* other than in exceptional circumstances. One such circumstance is primary hyperaldosteronism due to adenoma. Here the tumour tissue is capable not only of aldosterone synthesis but also of synthesizing significant quantities of cortisol [3]. This abnormal juxtaposition may account for the high 18-hydroxycortisol secretion rates in this syndrome. The effects of chronic ACTH treatment *in vivo* may further demonstrate the close relationship between cortisol and 18-hydroxycortisol biosynthesis. This treatment causes a transient rise in aldosterone secretion which rapidly returns to normal and then subnormal values. Cortisol secretion, however, remains consistently elevated; that of 18-hydroxycortisol also [33]. In chronically-stimulated animals, zona glomerulosa tissue is reduced or absent. In this situation, it again seems likely that 18-hydroxycortisol is synthesized in the zona fasciculata. Moreover, being larger, the steroid synthesizing capacity of the zona fasciculata should far exceed that of the zona glomerulosa *in vivo*.

A similar hypothesis may explain the even higher levels of 18-hydroxycortisol in dexamethasone suppressible hyperaldosteronism. It has been suggested that a single enzyme cytochrome *P*450 is responsible for aldosterone synthesis from DOC, that it occurs in both zona glomerulosa and zona fasciculata [42] but that it fails to operate in the latter zone due to pseudo-substrate inhibition (see [9]). It is further suggested that, in dexamethasone-suppressible hyperaldosteronism, some mutation of the enzymes makes it unresponsive to this inhibition. Abnormally high DOC levels [5] may be evidence of such a mutation. If this is the case, the enzymes would continue to function in a high cortisol environment.

Thus, the results of the current study, suggest that 18-hydroxycortisol is a product of zona fasciculata rather than either the zona glomerulosa or a restricted transition zone. When the zona glomerulosa (or other aldosterone-producing tissue) is exposed to cortisol, it is

capable of efficient 18-hydroxylation of this substrate. However, there are two important pieces of evidence which contradict this hypothesis. The first of these, the response to 18-hydroxycortisol secretion to sodium depletion, a zona glomerulosa characteristic, has already been mentioned. The second is a recent careful study in which 18-hydroxycortisol, cortisol and aldosterone secretion rates from serial slices of bovine adrenal gland were compared [46]. The capacity to secrete 18-hydroxycortisol was greatest in the outer slice, as was that for aldosterone, and fell progressively in later slices. The converse was true of cortisol (although cortisol was secreted from the outer slice). Cortisol and 18-hydroxycortisol therefore did not correlate positively. It is difficult at present to reconcile these findings with our own or with the role of cortisol as primary substrate.

The nature of the transition from zona glomerulosa to zona fasciculata function also remains unclear. Is there indeed a distinct transitional zone composed of cells capable of both functions or is the change of function sudden in individual cells but occurring in a small proportion of cells at a time, giving a zone of mixed rather than dual function? To answer this question, much more efficient methods of separation and characterization of cell types are necessary.

Acknowledgement—Our thanks are due to Miss Angela McKay for her invaluable aid in preparing this manuscript.

REFERENCES

1. Zajicek G., Ariel I. and Arber N.: The streaming adrenal cortex: direct evidence of centripetal migration of adrenocytes by estimation of cell turnover rate. *J. Endocr.* **111** (1986) 447–482.
2. Chu M. D. and Ulick S.: Isolation and identification of 18-hydroxycortisol from the urine of patients with primary aldosteronism. *J. Biol. Chem.* **257** (1982) 2218–2244.
3. Ulick S. and Chu M. D.: Hypersecretion of a new corticosteroid, 18-hydroxycortisol in two types of adrenocortical hypertension. *Clin. Exp. Hypert.* **4** (1982) 1771–1777.
4. Gomez-Sanchez C. E.: 18-Hydroxycortisol and 18-hydroxycortisone, steroids from the transitional zone. *Endocrine Res.* **85** (1984) 609–615.
5. Connell J. M. C., Kenyon C. J., Corrie J. E. T., Fraser R., Watt R. and Lever A. F.: Dexamethasone-suppressible hyperaldosteronism. Adrenal transition cell hyperplasia? *Hypertension* **8** (1986) 669–676.
6. Hamlet S. M., Gordon R. D., Gomez-Sanchez C. E., Tunny T. J. and Klemm S. A.: Adrenal zone transitional zone steroids, 18-oxo and 18-hydroxycortisol, useful in the diagnosis of primary aldosteronism, are ACTH-dependent. *Clin. Exp. Pharmac. Physiol.* **15** (1988) 317–322.
7. Corrie J. E. T., Edwards C. R. W. and Budd P. S.: A radioimmunoassay for 18-hydroxycortisol in plasma and urine. *Clin. Chem.* **31** (1985) 849–852.
8. Davis J. R., Burt P. S., Corrie J. E. T., Edwards C. R. W. and Sheppard M. C.: Dexamethasone-suppressible hyperaldosteronism: studies on overproduction of 18-hydroxycortisol in three affected family members. *Clin. Endocr.* **29** (1988) 297–308.
9. Fraser R.: Inborn errors of corticosteroid biosynthesis and metabolism: their effects on electrolyte metabolism and blood pressure. In *Clinical Aspects of Secondary Hypertension* (Edited by J. I. S. Robertson) Elsevier, Amsterdam, 2nd Edn. In press.
10. Gomez-Sanchez E. P., Gomez-Sanchez C. E., Smith J. S., Ferris M. W. and Foecking M.: Receptor binding and biological activity of 18-hydroxycortisol. *Endocrinology* **115** (1984) 462–466.
11. Asano K. and Harding B. W.: Biosynthesis of adrenodoxin in mouse adrenal tumour cells. *Endocrinology* **99** (1976) 977–987.
12. Connell J. M. C. and Fraser R.: Adrenal corticosteroid synthesis and hypertension. *J. Hypert.* In press.
13. Connell J. M. C., Whitworth J. A., Davies D. L., Lever A. F., Richards A. M. and Fraser R.: Effects of ACTH and cortisol administration on blood pressure, electrolyte and metabolism, atrial natriuretic peptide and renal function in normal man. *J. Hypert.* **5** (1987) 425–433.
14. Whitworth J. A., Hewitson T. D., Ming L., Wilson R. S., Scoggins B. A., Wright R. D. and Kincaid-Smith P.: Adrenocorticotrophin-induced hypertension in the rat: haemodynamic, metabolic and morphological characteristics. *J. Hypert.* **8** (1990) 27–36.
15. McDougall J. G., Butkus A., Coghlan J. P., Denton D. A., Miller J., Oddie C. J., Robinson P. M. and Scoggins B. A.: Biosynthetic and morphological evidence for inhibition of aldosterone production following administration of ACTH to sheep. *Acta Endocr.* **94** (1980) 559–570.
16. Fraser R., Buckingham J. C., Mason P. A. and Semple P. F.: The influence of sodium ACTH and angiotensin II on plasma corticosteroids in man: some implications in the control of corticosteroid biosynthesis. In *The Endocrine Function of Human Adrenal Cortex* (Edited by V. H. T. James, M. Serio, G. Giusti and L. Martini). Academic Press, London (1978) pp. 359–374.
17. Shepherd R. M., Fraser R., Nichols D. J. and Kenyon C. J.: Efflux of potassium ions in angiotensin II stimulated bovine adrenocortical cells. *J. Endocr.* **127** (1990) 297–304.
18. Spät A.: A stimulus-secretion coupling in angiotensin-stimulated adrenal glomerulosa cells. *J. Steroid Biochem.* **29** (1988) 443–453.
19. Dluhy R. G., Axelrod L., Underwood R. H. and Williams G. H.: Studies of the controls of aldosterone concentration in normal man. Effect of dietary potassium and acute potassium infusion. *J. Clin. Invest.* **51** (1972) 1950–1957.
20. Oelkers W., Brown J. J., Fraser R., Lever A. F., Morton J. J. and Robertson J. I. S.: Sensitization of the adrenal cortex to angiotension II in sodium deplete normal man. *Circ. Res.* **34** (1974) 69–77.
21. Hollenberg N. K., Chenitz W. R., Adams D. F. and Williams G. H.: Reciprocal influence of salt intake on adrenal zone glomerulosa and renal vascular responses to angiotensin II in normal man. *J. Clin. Invest.* **54** (1974) 34–42.
22. Drake C. R. and Carey R. M.: Dopamine modulates sodium dependent aldosterone responses to angiotensin II in humans. *Hypertension* **6** (Suppl. 1) (1984) 119–123.
23. Connell J. M. C., Tonolo G., Davies D. L., Finlayson J., Ball S. G., Inglis G. and Fraser R.: Dopamine affects

angiotensin II-induced steroidogenesis by altering clearance of the peptide in man. *J. Endocr.* **113** (1987) 139–146.
24. Connell J. M. C., Kenyon C. J., Ball S. G., Davies D. L. and Fraser R.: Dopamine effects on adrenocorticotrophin-stimulated aldosterone, cortisol, corticosterone and 11-deoxycorticosteroid concentrations in sodium-replete and sodium-deplete man. *J. Endocr.* **109** (1986) 339–344.
25. Ganguly A.: Dopaminergic regulation of aldosterone secretion: how credible? *Clin. Sci.* **66** (1984) 631–637.
26. Sowers J. R.: Dopamine regulation of renin and aldosterone secretion: a review. *J. Hypert.* **2** (Suppl. 1) (1984) 67–73.
27. Fraser R.: Dopamine regulation of aldosterone secretion: an open question? *J. Endocr.* **112** (1987) 179–181.
28. Cuneo R. C., Espiner E. A., Nicholls M. G., Yandle T. G. and Livesey J. H. Effect of physiological levels of atrial natriuretic peptide on hormone secretion: inhibition of angiotensin-induced aldosterone secretion and renin release in normal man. *J. Clin. Endocr. Metab.* **65** (1987) 765–772.
29. Douglas J. G.: Estrogen effects on angiotensin receptors are modulated by pituitary in female rats. *Am. J. Physiol.* **252** (1987) E57–E62.
30. Corrie J. E. T., Edwards C. R. W., Padfield P. L. and Budd P. S.: Factors affecting the secretion of 18-hydroxycortisol, a novel steroid of relevance to Conn's syndrome. *Clin. Endocr.* **23** (1985) 579–586.
31. Gomez-Sanchez C. E., Upcavage R. J., Zager P. G., Foecking M. F., Holland D. B. and Ganguly A.: Urinary 18-hydroxycortisol and its relationship to the excretion of other adrenal steroids. *J. Clin. Endocr. Metab.* **65** (1987) 310–314.
32. Gomez-Sanchez C. E., Zager P. G., Foecking M. F., Holland D. B. and Ganguly A.: 18-Oxocortisol: effect of dexamethasone, ACTH and sodium restriction. *J. Steroid Biochem.* **32** (1989) 409–412.
33. Gomez-Sanchez C. E., Clore J. N., Estep H. L. and Watlington C. O.: Effect of chronic adrenocorticotropin stimulation on the excretion of 18-hydroxycortisol and 18-oxocortisol. *J. Clin. Endocr. Metab.* **67** (1988) 322–366.
34. Ferris J. B., Brown J. J., Fraser R., Lever A. F. and Robertson J. I. S.: Primary aldosteronism excess: Conn's syndrome and similar disorders. In *Handbook of Hypertension 2: Clinical Aspects of Secondary Hypertension* (Edited by J. I. S. Robertson). Elsevier, Amsterdam (1983) pp. 132–161.
35. Fraser R., Beretta-Piccoli C., Brown J. J., Cumming A. M. M., Lever A. F., Mason P. A., Morton J. J. and Robertson J. I. S.: Response of aldosterone and 18-hydroxycorticosterone in normal subjects and patients with essential hypertension, Conn's syndrome and nontumerous hyperaldosteronism. *Hypertension* **3** (Suppl. 1) (1981) I-87–I-92.
36. Fraser R., Mason P. A. and Young J.: The acute effect of angiotensin II on adrenal and anterior pituitary function in normal subjects and subjects with primary hyperaldosteronism. *Prog. Biochem. Pharmac.* **17** (1980) 14–19.
37. Semple P. F., Buckingham J. C., Mason P. A. and Fraser R.: Suppression of plasma ACTH concentrations by angiotensin II infusion in normal humans and in a subject with a steroid 17α-hydroxylase defect. *Clin. Endocr.* **10** (1979) 137–144.
38. Rayyis S. S. and Horton R.: Effect of angiotensin II on adrenal and pituitary function in man. *J. Clin. Endocr. Metab.* **32** (1971) 539–546.
39. Ramsay D. J., Keil L. C., Sharpe M. C. and Shinsako J.: Angiotensin II infusion increases vasopressin, ACTH and 11-hydroxycorticosteroid secretion. *Am. J. Physiol.* **234** (1978) R66–R71.
40. Haning R., Tait S. A. S. and Tait J. F.: *In vitro* effects of ACTH, angiotensins, serotonin and potassium on steroid output by isolated adrenal cells. *Endocrinology* **87** (1970) 1147–1167.
41. Kenyon C. J., Young J. and Fraser R.: Potassium fluxes in bovine adrenal cells during adrenocorticotropin stimulation. *Endocrinology* **116** (1985) 2279–2285.
42. Yanagibashi K., Haniu M., Shively J. E., Shan W. H. and Hall P.: The synthesis of aldosterone by the adrenal cortex. Two zones (fasciculata and glomerulosa) possess one enzyme for 11β 18-hydroxylation. *J. Biol. Chem.* **261** (1986) 3556–3562.
43. Ogishima T., Mitani F. and Ishimura Y.: Isolation of aldosterone synthase cytochrome *P*450 from zona glomerulosa mitochondria of rat adrenal cortex. *J. Biol. Chem.* **264** (1989) 10935–10938.
44. Lauber M., Böni-Schnetzler M. and Müller J.: Potassium raises cytochrome $P450_{11\beta}$ mRNA level in zona glomerulosa of rat adrenals. *Molec. Cell. Endocr.* **72** (1990) 159–166.
45. Imai, Shimada H., Okada Y., Matsushima-Hibiya Y., Ogishima T. and Ishimura Y.: Molecular cloning of a cDNA encoding aldosterone synthase cytochrome *P*-450 in rat adrenal cortex. *FEBS Lett.* **263** (1990) 299–303.
46. Gomez-Sanchez C. E., Ferris M. W., Foecking M. F. and Gomez-Sanchez E. P.: Synthesis of 18-hydroxycortisol and 18-oxocortisol in bovine adrenal slices. *J. Steroid Biochem.* **33** (1989) 595–598.